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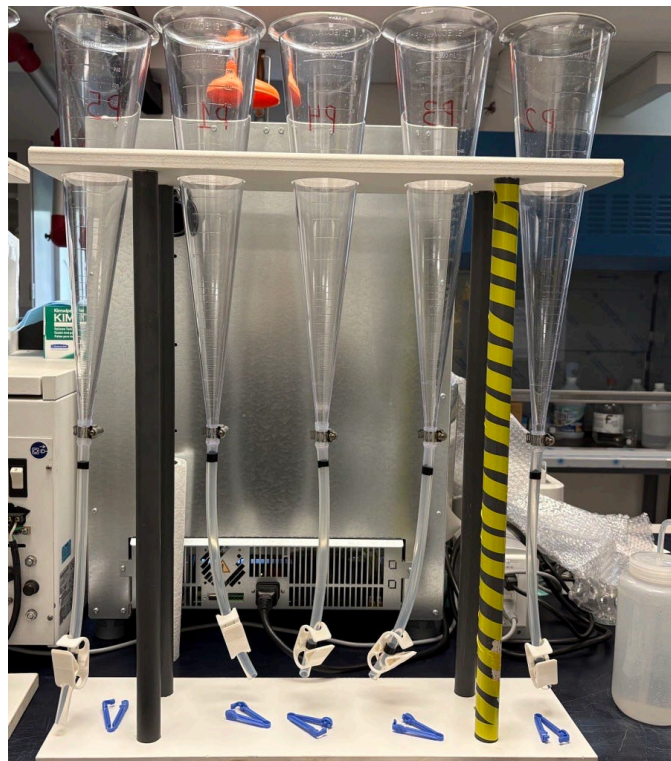
Final Report No. ST-2025-22058-01

## **Optimization of Sample Analysis Methods for the Early Detection of Invasive Dreissenid Mussels in Reclamation Reservoirs**

Research and Development Office

### **Science and Technology**

Research Program



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14. ABSTRACT Early detection of invasive mussels remains a critical priority for the Bureau of Reclamation, as rapid establishment can cause severe operational and economic impacts. Current laboratory methods rely on microscopy for detection of veliger larvae collected from plankton tows and concentrated water samples. While effective, these methods are labor-intensive and sensitive to inefficiencies in sample handling and subsample collection. Four main aspects of sample handling and detection were examined: 1) optimization of subsample collection after sample settlement, 2) review of external laboratory SOP's, 3) exploration of automated imaging technologies, and 4) preliminary evaluation of alternative detection methods based on volatile compound analysis. Relative to current methods, utilizing a modified venoset and 1/4-inch interior diameter tubing at the base of the sample settlement cone, a 3/8-inch interior diameter silicone tubing reduced cone construction and repair needs, improved setup and takedown efficiency, decreased spill frequency, and significantly lowered material and labor cost. Scoping investigations into automated imagery of samples and detection of veliger scent in samples found promising results and leads that warrant further investigation. Initial studies have shown that canines can detect adult mussels and veligers in plankton tow samples containing ethanol and electronic scent detection technologies should be a focus of future testing.					
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**Cover Image** – Imhoff settling cones for larval invasive mussel detection, with tubing and clamps attached (Bureau of Reclamation).



# **Optimization of Sample Analysis Methods for the Early Detection of Invasive Dreissenid Mussels in Reclamation Reservoirs**

**Final Report No. ST-2025-22058-01  
EcoLab-F587A-2025-06**

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# Peer Review

## **Bureau of Reclamation Research and Development Office Science and Technology Research Program**

**Final Report ST-2025-22058-1  
EcoLab-F587A-2025-06**

### **Optimization of Sample Analysis Methods for the Early Detection of Invasive Dreissenid Mussels in Reclamation Reservoirs**

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This document has been reviewed under the Research and Development Office Discretionary peer review process, consistent with Reclamation Policy CMP P14. It does not represent and should not be construed to represent the Bureau of Reclamation's determination, concurrence, or policy.



# Acronyms and Abbreviations

3D	three-dimensional
CPLM	Cross Polarized Light Microscopy
CPN	Columbia-Pacific Northwest Region
DI water	Deionized water
DNA	Deoxyribonucleic acid
eDNA	Environmental DNA
EtOH	Ethanol
ID	Interior diameter
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
Reclamation	Bureau of Reclamation
RISL	Reclamation's Invasive Species Lab
SOP	Standard Operating Procedure
TSC	Technical Service Center
TSS	Total Suspended Solids
WRP	Western Regional Panel

## Measurements

cm	centimeter
L	liter
M	meter
mL	milliliter
mm	millimeter
μm	micron/micrometer/one-thousandth of a millimeter

## Symbols

“	inch
‘	feet/foot
%	percent
≈	approximation of value
°C	degrees Celsius
<	less than



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# Executive Summary

Early detection of invasive mussels remains a critical priority for the Bureau of Reclamation, as rapid establishment can cause severe operational and economic impacts. Current laboratory methods rely on microscopy for detection of veliger larvae collected from plankton tows and concentrated water samples. While effective, these methods are labor-intensive and sensitive to inefficiencies in sample handling and subsample collection. This study, conducted under the Science and Technology Program, evaluated alternative approaches to optimize subsample collection and improve overall monitoring efficiency.

Four main aspects of sample handling and detection were examined: 1) optimization of subsample collection after sample settlement, 2) review of external laboratory SOP's, 3) exploration of automated imaging technologies, and 4) preliminary evaluation of alternative detection methods based on volatile compound analysis. The results presented here focus primarily on subsample collection methods.

Testing revealed that use of a custom 3D-printed coupler with an integrated shutoff valve attached to the bottom of the Imhoff cone retained residual water containing veligers, reducing recovery rates and creating cross-contamination risks. Using Imhoff cones that drain with a wider tubing measuring 3/8-inch interior diameter (ID) provided more consistent results. Latex tubing (3/8 in ID) supported reliable recovery but is susceptible to degradation over time due to repeated exposure to acetic acid for purposes of decontamination, limiting its practical use. Silicone tubing with an ID of 3/8-inch consistently delivered high veliger recovery while offering superior chemical stability, ease of handling, and reduced risk of clogging compared to existing venoset systems. Relative to current methods, utilizing a modified venoset and 1/4-inch ID tubing at the base of the cone, the 3/8-inch ID silicone tubing reduced cone construction and repair needs, improved setup and takedown efficiency, decreased spill frequency, and significantly lowered material and labor costs.

An exploratory trial of subsample collection directly from settled material within sample bottles confirmed successful veliger recovery through pipetting. However, operator variability has the potential to introduce errors, limiting immediate application of this method under routine monitoring.

Review of external SOPs confirmed that many laboratories employ similar settlement and collection techniques, with some variation in filtration and chemical treatment. Several additional methods were identified (e.g., glycerin, sugar solutions, vibration techniques) but were deemed impractical for Reclamation's monitoring needs due to sample size and debris load.

Scoping investigations into automated imagery of samples and detection of veliger scent in samples found promising results and leads that warrant further investigation. A Material Transfer Agreement was set up with a company developing a device that utilizes machine learning to train a camera to detect veligers in water samples. The partnership was successful in that the lab was

able to provide guidance and training samples to facilitate development of the technology. The company successfully applied to another Reclamation grant opportunity allowing continuation of the effort.

The investigation into the detection of veliger scent in early detection samples built from two previously funded Reclamation Research Office funded efforts. The lab partnered with Mussel Dogs, a canine training company, to investigate canines to detect veligers in samples preserved with ethanol. In the initial studies the trained dogs were able to detect adult mussels in plankton tow samples preserved with ethanol, but they could not detect veligers. A follow up study found that untrained dogs were able to be trained to detect veligers in ethanol. The results of this study suggest that canines may be able to be used to scan early detection samples alongside microscopy or genetic analysis to assist with identifying positive samples. Additional studies will be conducted as part of a continuing S&T Project.

This study also followed up on a Technology Search completed to identify existing technologies for electronic scent detection. The search identified seven companies with promising technologies and the Invasive Species Lab reached out to all seven. The effort resulted in a single company of interest, and a future scoping study will be pursued.

Overall, this study demonstrates that simple modifications to existing laboratory procedures can significantly improve both reliability and efficiency of veliger detection. Silicone tubing with a 3/8-inch ID in particular shows strong potential for adoption into Reclamation's invasive mussel monitoring protocols, with immediate benefits in cost savings, reduced labor demands, and improved operational consistency. Further validation across diverse sample types and field conditions is recommended, along with continued exploration of automated imaging and alternative scent detection technologies.

# 1.0 Introduction

Invasive mussel fouling poses a significant operational risk to Reclamation facilities by reducing flow capacity, impairing equipment functionality, and increasing the likelihood of unplanned outages and costly maintenance. Documented impacts include elevated frequencies of high-temperature alarms at pumping and hydropower plants due to biofouling of cooling systems, head loss and reduced capacity from fouled intake structures, and complete inoperability of small-diameter systems such as fire suppression lines caused by accumulation of shell debris.

The Bureau of Reclamation's Invasive Species Lab (RISL, previously Ecological Research Lab or EcoLab) processes plankton tow samples from water bodies across the western U.S. to determine the presence of mussel veligers. Standard analyses employ cross-polarized light microscopy (CPLM) and confirmatory quantitative polymerase chain reaction (qPCR), which state partners require before designating a waterbody as invasive mussel-positive.

RISL typically receives more than 2,000 samples for analysis annually. These samples vary widely in composition, often containing sediment, zooplankton, phytoplankton, algae, and other materials that exhibit birefringence under CPLM. Complete microscopic examination of the entirety of each sample (volumes typically 200–500 mL) is not practical, and volume-reduction techniques are utilized to improve processing efficiency while maintaining detection sensitivity.

Microscopic analysis of plankton tow samples is labor-intensive and time-consuming. The current standard operating procedure (SOP) involves concentration of sample particulates prior to microscopic observation. Each sample is transferred to an Imhoff cone (Figure 1) and allowed to settle undisturbed for 24 hours. The heaviest 15 mL of settled material is then collected as the working subsample for cross-polarized light examination. This settling procedure reduces the total volume required for analysis and increases the likelihood of detecting invasive mussel veligers, due to the weight of their calcium carbonate shells and the likelihood of them settling at the bottom.

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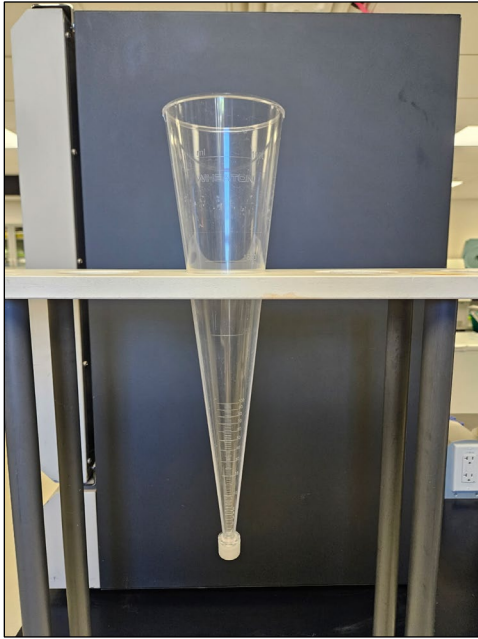


Figure 1.—Imhoff settling cone, 1 L volume

Previous evaluations of this protocol have demonstrated that total veliger recovery is often incomplete; all veligers present in the bulk sample do not consistently transfer into the concentrated subsample. Identifying the cause of veliger loss has proven challenging, with high variability in recovery even when using clear water samples and rigorous transfer/rinsing methods. Despite this limitation, testing has shown that the probability of false-negative results remains very low (< 2%).

The objective of the present study is to increase the efficiency of water-sample processing and analysis for detection of invasive mussel veligers by evaluating alternative or novel methods that address persistent challenges. The goal is to refine and, where appropriate, integrate new techniques into RISL's SOP to improve processing efficiency without compromising sensitivity. The anticipated outcomes will enhance Reclamation's capacity to process larger volumes of samples at reduced cost, improve early-detection capability for new infestations, and strengthen rapid response efforts to protect western water resources and infrastructure.

This study focused on four key areas of water-sample handling and invasive mussel veliger detection:

1. Optimization of subsample collection – evaluation of methods for collection of the heaviest 15 mL to improve recovery efficiency.
2. Review of external laboratory methodologies – assessment of alternative veliger monitoring protocols currently in use by other laboratories.

3. Evaluation of automated imaging technologies – investigation of image-based systems for veliger detection and enumeration.
4. Exploration of volatile compound-based detection – preliminary assessment of whether mussel veligers can be identified through volatile chemical signatures (i.e., scent detection).

## 1.1. Previous Work & Study Question

The Bureau of Reclamation's Science & Technology Program has supported multiple studies aimed at improving detection, prevention, and control of invasive mussels. Prior work relevant to early detection and veliger monitoring includes:

Detection of environmental DNA (eDNA) by polymerase chain reaction (PCR) and additional laboratory capabilities have advanced methods for identifying mussel presence via genetic markers.

Other previous projects have tested the effects of different preservative and storage strategies on veliger detection via microscopy and qPCR, as well as morphological studies developing an understanding of veliger structure to improve taxonomic identification.

Scoping level projects have investigated the use of canines and electronic sensing devices to detect volatile compounds associated with invasive mussels. These investigations uncovered that scent detection has significant potential to provide another method for veliger detection.

These efforts have improved sample handling, species-level identification, and established base protocols for microscopy and molecular tools. However, questions remain in 1) ensuring recovery of veligers from large and complex sample matrices, 2) reducing labor/time in microscopy, 3) reducing variation and false negatives, and 4) assessing novel or automated detection methods (imaging and volatile detection).

## 2.0 Methods

### 2.1. Subsample Collection Optimization

Optimization of subsample collection after settlement focused on improving the transfer of the settled fraction from Imhoff cones to 15 mL analysis tubes. Three primary approaches were evaluated:

- Custom 3D-printed coupler with integrated shutoff valve – designed to allow settlement directly into 15 mL conical collection tubes, eliminating intermediate transfer steps.

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- Wide tubing-based collection systems – 3/8-inch ID latex and silicone tubing (secured with ring clamps at the cone outlet) terminated with two pinch-type clamps to regulate flow into collection tubes.
- Settlement within the sample bottles – subsamples collected from the bottom of the sample bottle via pipetting.

Performance of each method was evaluated based on mussel veliger recovery as well as considerations of labor, materials, durability, and overall potential to improve sample processing efficiency. An additional consideration is consistency of collection techniques amongst RISL staff and interns.

### 2.1.1. Sample Preparation

Test samples were prepared using combinations of deionized (DI) water and ethanol-preserved field samples collected from western U.S. waterbodies that had already been analyzed and determined to be negative for presence of veligers. All samples were treated with a 7.5 pH buffer to minimize degradation of veligers spiked into the samples. Known concentrations of veligers were introduced (spike-in trials) to enable quantitative assessment of recovery efficiency.

To minimize cross-contamination, all containers and equipment were new or were decontaminated in a vinegar bath and rinsed thoroughly between trials. When possible, equipment was visually inspected with CPLM prior to reusing.

### 2.1.2. Sample Settlement

Trials utilizing Imhoff settling cones were set up on racks with collection apparatuses (3/8-inch ID tubing or 3D-printed attachments) secured to the narrow end of each cone (Figures 2 and 3). Water samples were transferred into cones, spiked with known numbers of veligers (typically 10-50 in each sample) and then allowed to settle undisturbed for a maximum of 24 hours. Original sample bottles were rinsed thoroughly with DI water into the cones to ensure complete transfer of particulates, and veligers were spiked directly into the cones after the samples were poured in. Contents were stirred to ensure homogenization of the sample.

The existing SOP configuration utilizes Imhoff cones with subsample collection from a modified medical venoset with attached 1/4-inch ID tubing at the end of the cone, equipped with roller clamps to control flow of the settled fraction into a 15 mL tube. The alternative Imhoff cone collection systems were evaluated against this baseline. (Figure 4)

Settlement within the sample bottles was conducted by setting sample bottles in a rack constructed to hold the bottles at a 45-degree angle. A 15 mL pipette was used to draw the subsample from the bottom of the bottle. Two pipetting methods were evaluated, including pulling directly from the lowest apex of the bottle as well as while moving the tip of the pipette in a sweeping motion through the settled particulates.

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Figure 2. —An Imhoff settling cone with 3/8-inch ID tubing attached, marked to denote where to add clamps to collect 15 mL volume from settled sample.



Figure 3. —An Imhoff settling cone with 3D printed coupler attached to a 15 mL collection tube.

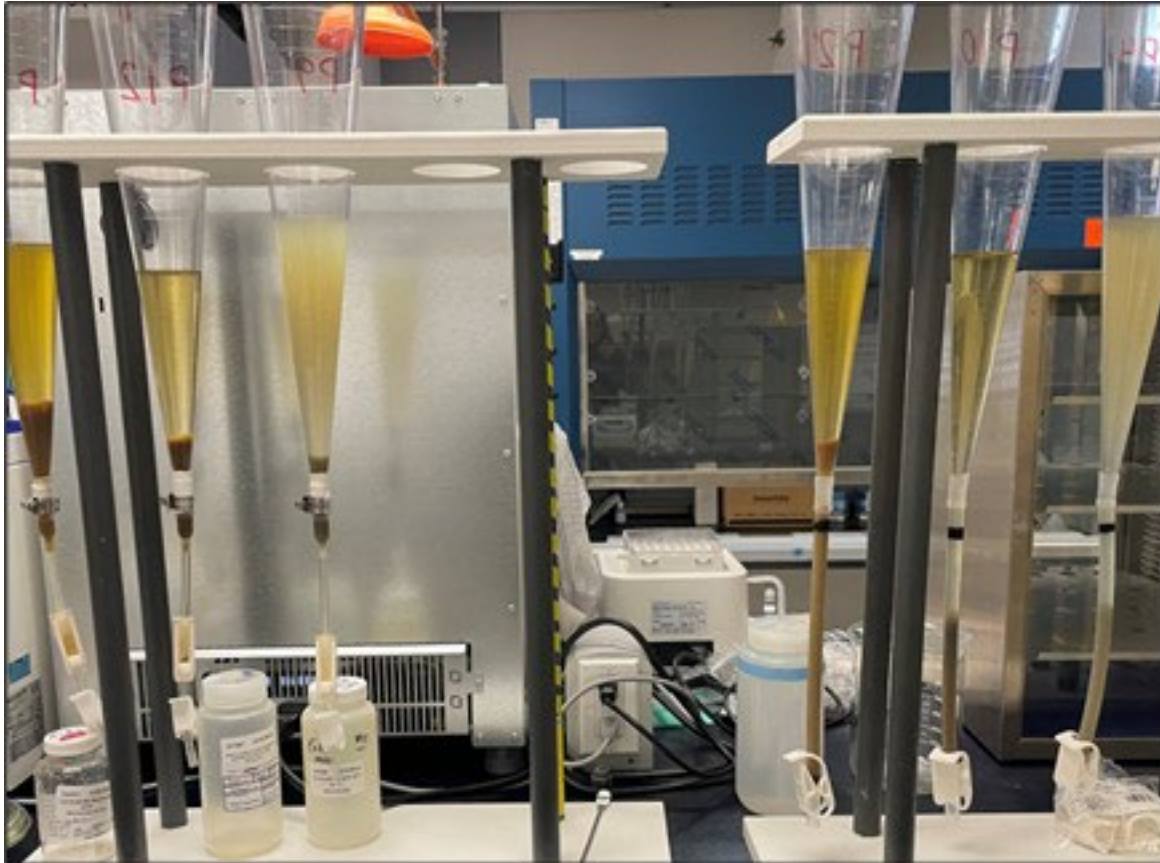


Figure 4. —Imhoff settling cones: Three with attached venosets and 1/4-inch ID tubing (left), and three with 3/8-inch ID tubing (right).

### 2.1.3. Settlement Collection Assessment

Veliger recovery from each configuration was quantified following the current RISL SOP using CPLM. Performance metrics included:

- Recovery efficiency – proportion of spiked veligers successfully transferred into subsamples, incidence of loss during transfer.
- Operational efficiency – setup time, ease of transfer, potential for sample loss or contamination, and cleanup requirements.
- Durability and reliability – frequency of leaks, clamp failure, or equipment breakage; maintenance needs; overall waste generation.
- Cost considerations – relative material and fabrication costs, reusability of components, and staff time required for operation and construction/repairs.

Results from these assessments were compared across configurations to identify subsample collection methods that maximize veliger recovery while reducing time, labor, and long-term operational costs.

## 2.2. Assessment of Standard Operating Procedures from External Laboratories

SOP documents were obtained from external laboratories that conduct larval mussel detection analyses. Each SOP was reviewed to identify guidance related to settlement, collection, and concentration techniques. A comparative spreadsheet was compiled to systematically evaluate procedural elements across institutions (Appendix A). Laboratories employing the same protocol as RISL were excluded to focus the review on alternative approaches.

In many cases, SOPs were adapted from existing, publicly available protocols rather than developed independently. Several unique or less commonly applied methods were identified during the review, including the use of glycerin or sugar solutions as density gradients, vibration techniques to dislodge or concentrate veligers, and specialized filtration methods. These approaches were not tested in this study due to logistical constraints (e.g., high sample volumes, debris loads, and processing throughput requirements), which would limit their practicality for routine monitoring.

The SOPs reviewed included:

- Reclamation Standard Operating Procedure: Preparation and Analysis of Water Samples for Dreissenid Mussel Veliger Detection: Microscopy (2010; updated 2022)
- Western Regional Panel on Aquatic Nuisance Species (2020)
- Monitoring for Invasive Mussels in Alberta's Irrigation Infrastructure: 2017 Report
- British Columbia Zebra and Quagga Mussel Early Detection and Rapid Response Plan (2015)
- California Department of Fish and Wildlife, Quagga/Zebra Mussel Plankton Tow Sampling Protocol (2021)
- Montana Fish, Wildlife, and Parks Quagga/Zebra Mussel Plankton Tow Sampling Protocol (2019)
- Early detection of dreissenid species: Zebra/Quagga mussels in water systems (2010)

## **2.3. Automated Image Analysis**

This study also continued efforts to investigate automated image analysis to be utilized for select samples. Monitoring samples that contain large numbers of veligers require a lot of time for analysis, and the use of an automated sampling system would have the potential to significantly reduce the amount of time it takes to analyze those samples, allowing lab staff to focus on early detection sample analysis. There are several automated sampling devices available on the market that are designed to monitor zooplankton and macroinvertebrates. Reclamation previously ran a prize challenge to identify such devices; however, most of the devices are designed to monitor populations in-situ and not in the lab and have not been designed specifically to detect veligers.

In 2020, RISL initially established a Material Transfer Agreement (MTA) with a company working to develop another automated sampling device that incorporates machine learning to specifically identify dreissenid mussel veligers. A MTA was pursued with this company because the prototype device was able to be utilized both in-situ and in the lab and was able to process samples quickly and without filtration. During the initial MTA in 2020, the company ran into some setbacks with development stemming from the pandemic and disruption in the supply chain. But an additional MTA was established because they had made progress with training the device and are working with a university and the United States Geological Survey (USGS) to do some in-situ testing with zebra mussel veligers in Minnesota.

During this investigation, Invasive Species Lab staff participated in a demonstration of the automated detection system capabilities. Lab staff provided guidance and confirmation of the system output. The Lab sent another set of training samples spiked with know quantities of veligers. The Lab also offered to prepare sets of samples containing veligers of each size class and other organisms that look like dreissenid veligers to be used in training the machine learning device.

## **2.4. Volatile Compound Detection**

### **2.4.1. Canine Detection Studies**

The results of a Science and Technology funded literature review suggested that detection of invasive mussel volatile compounds is a promising area of study and should be considered as a method for invasive mussel early detection. RISL is interested in utilizing canines to scan samples before or after analysis by microscopy and/or qualitative polymerase chain reaction (qPCR) to provide an initial or secondary scan of samples to detect samples that may contain mussel veligers. This method would be especially helpful for samples that are difficult to analyze, including samples containing large amounts of zooplankton, phytoplankton, and sediment.

Canines have highly developed olfactory senses which are up to 100,000 times more sensitive than humans. Detection dogs have been trained to sniff out explosives, drugs, and even cancer in human blood samples. Dogs have been used to detect adult invasive mussels at watercraft

inspection stations for many years. Several studies have also investigated the ability of dogs to detect the microscopic veliger stage of mussels. The ability of dogs to detect mussels in preserved samples is unknown because it is unclear if the scent pattern would be disrupted or changed by alcohol.

This scoping study was conducted in collaboration with Mussel Dogs, a canine training company based in CA that trains dogs to detect invasive mussels on boats. The study focused on the ability of canines to detect veligers in ethanol preserved samples. The first phase of the investigation was to determine if canines can detect adult mussels preserved in ethanol. Dead frozen adult quagga mussels were added to lake water plankton tow samples preserved in ethanol. Reclamation sent a variety of actual early detection samples collected at reservoirs across the western United States. The samples were from invasive mussel-negative water bodies and had been analyzed to confirm that they did not contain veligers.

The second phase of the study was to determine if the canines (which had already been trained to detect live veligers) could detect and identify samples of 1000 veligers in plankton tow samples preserved in ethanol.

The third phase was to determine if untrained canines can be trained to detect veligers in lake water samples preserved in ethanol. Two canines that had never been exposed to adult mussels or veligers were exposed to preserved plankton tow samples containing 1000 and 500 veligers.

#### **2.4.2. Scent Detection Sensor Technologies**

While canine scent detection is a potential option for laboratory use, there are still some limitations. The development of scent detection sensors could prove to be as effective or more effective as canines while providing additional benefits. The Reclamation Research and Development Office Prize Competition Program previously supported a Technology Search, to gather information about the potential to develop scent sensors for the detection of dreissenid mussels. The scope of the technology search was to identify current and emerging scent detection sensor technologies that could be utilized to detect dreissenid mussel veligers and possibly their eDNA in plankton tow samples that contain other zooplankton, phytoplankton, sediment, lake water, ethanol, and tris buffer.

The technology search identified 7 promising technologies. For this study, we followed up with the seven companies and discussed the following questions.

- Is it possible to utilize the technology for veliger detection?
- How well would the technology work on complex samples containing other zooplankton, phytoplankton, ethanol, and tris buffer?
- What would development look like for teachable sensors or custom development?
- What is the estimated cost of development?
- Is there interest in working with Reclamation, and how would Reclamation be involved in development?
- What would be the estimated cost to purchase the technology after development?

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- What is the timeline of development to deployment?
- Could the technology be customized for use on other invasive species or algal toxins?
- What limitations exist?

### 3.0 Results

#### 3.1. Settlement Collection Method

Evaluation of alternative subsample collection methods demonstrated distinct differences in operational efficiency and long-term feasibility. The custom 3D-printed coupler with integrated shutoff valve was functional but operationally problematic. During trials, veligers were detected in residual water retained between the valve mechanism and the collection tube, indicating incomplete transfer of the settled fraction. This residual retention not only reduced recovery efficiency but also increased the potential for cross-contamination between samples. In practice, the coupler created additional cleanup challenges and was not considered suitable for routine use.

Latex tubing with 3/8-inch ID affixed to the base of the Imhoff cone provided more reliable transfer. Veligers were successfully recovered in both clear-water trials and samples with high suspended solids and zooplankton content. The tubing attached directly to the cone with simple clamps allowed consistent flow control and minimized sample loss. However, repeated exposure to acetic acid, routinely used for decontamination of laboratory equipment, would eventually cause deterioration of the latex material, reducing durability and increasing replacement frequency.

Silicone tubing with a 3/8-inch ID performed comparably to latex in terms of veliger recovery, with consistent detection across both low and high-debris sample matrices. Importantly, silicone has superior chemical stability and was easier to manipulate during setup and transfer. These advantages translated into improved reliability and reduced maintenance demands, making silicone tubing the most promising candidate for incorporation into RISL's Laboratory SOP. Utilization of the silicone 3/8-inch ID tubing was inspired by practices utilized by Reclamation's CPN Water Lab.

Veliger recovery was also confirmed from the subsample when pipetted from settled material within the sample bottle. Recovery rates did not differ among the pipetting techniques tested, although it was noted that results had the potential to be highly sensitive to operator technique. This method was not adopted for routine use but may warrant further evaluation.

Relative to the previously used venoset and 1/4-inch ID tubing with roller clamps, Imhoff cones with the wider silicone tubing configuration provided multiple benefits. A 13-inch distance of the 3/8-inch ID tubing holds 15 mL. Clamping the tubing at the top and bottom of this measured and marked length of tube allows for collection of the exact desired amount. Since the implementation of this design, no repairs have been required, compared to the need to repair 5-6 cones per month with the original design. Setup was faster ( $\approx 33\%$ ) while takedown was

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significantly more efficient ( $\approx 50\%$ ) due to reduced clogging at the cone outlet and lower spill potential. It was noted that the 3/8-inch ID silicone tubing was easier to learn and operate, reducing the training curve for new personnel and interns. Cleaning requirements were also reduced, with significantly fewer spills occurring while collecting the fraction of the sample directly from the tube. Importantly, the silicone configuration effectively eliminated critical failures previously associated with clog removal and the tendency for particulates to stay contained in the shoulder of the venoset (Figure 5). When tests were being conducted on the wider latex or silicone tubing, use of a roller clamp appeared to crush veligers that settled in the crevice of the tubing above the roller. Crushed veligers were not observed after switching to pinch-style clamps. Material and labor costs were substantially lower, with overall reductions in cone assembly, consumables, and waste management estimated at  $\approx 51\%$ .

Table 1. —Sample handling time comparisons between Imhoff cone tubing types.

Imhoff Tubing Type	Sample Setup Time (50 samples)	15 mL Collection Time	Repairs
Venoset & 1/4" ID Tubing	90 minutes	120 minutes	30 minutes
3/8" ID Tubing	60 minutes	60 minutes	0 minutes

Table 2. — Construction cost comparisons between types of Imhoff cone designs.

Material for 75 Settling Cones	Venoset & 1/4" ID Tubing	3/8" ID Tubing
75 venosets	\$446	N/A
100' tubing	\$218	\$330
Plumbers tape	\$6	N/A
<b>Total</b>	<b>\$670</b>	<b>\$330</b>



Figure 5. —A venoset with particulates retained in the shoulder after the 15 mL sample was collected.

### **3.2. SOP Review**

The comparative review of seven standard operating procedures (SOPs) from U.S. and Canadian agencies revealed a moderate degree of variability in sampling, preservation, and processing methods for dreissenid veliger detection. Most protocols specified the use of 63–64 µm plankton nets, with sampling strategies adapted to waterbody size and depth. Several SOPs recommended a minimum of 1,000 liters of water filtered per site, though this varied in turbid or debris-heavy conditions.

Preservation methods were generally consistent, with most protocols using 70% ethanol by volume, often buffered to maintain pH. However, concentrations and buffering agents varied, and one protocol included live sample handling. Cold storage during transport and prior to analysis was commonly recommended, though not universally specified.

Only two SOPs (Reclamation and the U.S. Geological Survey) provided detailed guidance on settling procedures, including the use of modified Imhoff cones and 24-hour settling times. Most other protocols did not describe settling or subsampling steps, with some relying on filtration-based methods or outsourcing analysis to external laboratories.

Cross-contamination prevention practices ranged from detailed decontamination protocols (e.g., vinegar or bleach soaks, dedicated nets) to no mention at all. Similarly, management of sediment or planktonic organisms was inconsistently addressed, with only a few SOPs providing guidance. Microscopy using cross-polarized light was referenced in most SOPs, and several included or referenced the use of PCR for genetic confirmation. One SOP also explored flow cytometry and dye-based techniques, though these are not widely utilized.

### **3.3. Automated Image Analysis**

The automated imaging company was unable to make progress without additional funding sources. They applied for funding through the Reclamation Invasive Mussel Spend Plan call for proposals and were selected for funding. The research and development are continuing in collaboration with a university. The Invasive Species Lab offered to continue to provide guidance and training samples if progress is made and will review the final report and follow up with the company if the resulting product is promising.

## 3.4. Volatile Compound Detection

### 3.4.1. Canine Detection Studies

It was determined that the canines had no issue detecting the adult mussels in the plankton tow, lake water samples that contained 70% ethanol. The canines consistently and easily detected samples that contained adult mussels and avoided samples that did not contain mussels. When the same mussel trained dogs were exposed to the ethanol preserved plankton tow samples containing 1000 veligers they could not detect the samples that contained veligers. Since the trained canines could not detect the veligers in preserved samples the trainers decided to train dogs that had not previously been trained to detect veligers. Those newly trained dogs were able to be trained to detect concentrations of 1000-500 veligers in ethanol preserved plankton tow samples.

Continued research with Mussel Dogs is planned in order to determine the lowest numbers of veligers that canines can detect in ethanol preserved samples. The trainers believe there may be a specificity difference between canines suggesting the need for exposure to veligers under a variety of conditions at the 1000 -500 level before reducing concentrations.

### 3.4.2. Scent Detection Sensor Technologies

Only one of the seven companies that were contacted provided a promising lead. The company offered to allow rental of their sensor equipment at a reasonable cost. The sensor can be trained with exposure to the scent of interest.

## 4.0 Discussion

This study demonstrated that alternative subsample collection methods can substantially influence operational efficiency in routine mussel veliger monitoring. The 3D-printed coupler with integrated shutoff valve, while conceptually appealing, introduced operational challenges that outweighed its potential benefits. Residual water retained within the valve assembly consistently contained veligers, reducing recovery efficiency and creating risk of cross-contamination between samples. In practice, the coupler design was also more difficult to clean and maintain, limiting its suitability for adoption in high-throughput laboratory operations.

Tubing-based methods that utilize tubing with an ID of 3/8-inch provided better performance and benefits over the venoset with 1/4-inch ID tubing, which was prone to clogging with organic and inorganic material like zooplankton, algae, or sediment, commonly present in concentrated samples collected from lakes and reservoirs. The 3/8-inch ID latex tubing enabled consistent transfer of settled material and successful recovery of veligers in both clear and debris-rich samples. However, its susceptibility to degradation when repeatedly exposed to acetic acid resulted in limited durability. This long-term chemical incompatibility would necessitate

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frequent replacement and increase long-term costs and waste. Latex also has the potential to pose a safety risk to staff that may have allergies.

Silicone tubing performed comparably to latex in terms of veliger recovery but offered distinct advantages in chemical stability, handling, and reliability. Silicone was unaffected by acetic acid exposure, easier to manipulate during setup and takedown, and reduced failure modes associated with clogging at the cone outlet. Relative to the venoset system currently in use with 1/4-inch tubing, the 3/8-inch silicone tubing attached directly to the cone decreased construction and repair requirements, shortened setup and takedown times, reduced spill frequency, and minimized the risk of critical failures. Cost analyses indicated substantial reductions in materials, labor, and waste generation, reinforcing silicone tubing as the most operationally feasible option for incorporation into future SOPs (Tables 1 and 2).

Since incorporation of the new design, RISL staff have been able to increase weekly sample processing from 50 to 60 samples, and weekly repairs of cones due to damaged tubing or venosets caused by clogging are no longer necessary. The lab is also using fewer cleaning supplies and spending less time cleaning spills that were frequent when using the narrow tubing with roller clamps. The optimized design and methods identified in this study have been incorporated into the RISL SOP for Laboratory Analysis of Invasive Mussel Early Detection Samples.

An exploratory evaluation of subsample collection directly from settled material within sample bottles demonstrated that veliger recovery was possible using pipetting techniques. However, this method has the potential for operator variability to introduce significant inconsistencies, with even subtle differences in pipetting approach influencing results. Because this method carries a high risk of operator-induced error, it is not recommended for immediate implementation. Nonetheless, its simplicity suggests it could warrant further testing to determine whether standardized training or automation could mitigate variability.

Collectively, these findings suggest incremental adjustments to sample handling procedures can yield meaningful improvements in both efficiency and reliability. While microscopy will remain labor-intensive, optimizing upstream handling steps can expand laboratory capacity by reducing staff time, material costs, and failure rates without compromising detection sensitivity.

## 5.0 Next Steps

Future work should build on the results of this study to further validate silicone tubing performance across a wider range of field conditions and sample types, and by more strategic examination of alternative approaches such as in-bottle-settlement pipetting under control and standardized protocols. Initial tests looking at the length of time required for samples to fully settle indicate the current protocol for 24-hour settling could potentially be reduced. Reducing the amount of time samples need to settle could allow for an increase in the number of samples processed on a weekly basis. Further integration of these optimized methods into RISL's SOPs will provide Reclamation with a more efficient, scalable, and cost-effective foundation for invasive mussel monitoring and rapid response.

## 6.0 References

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## 7.0 Glossary

**Dreissenid (quagga/zebra) mussel.** (*Dreissena rostriformis bugensis*) and zebra (*Dreissena polymorpha*) are freshwater bivalve mussels native to Eurasia. Established as an invasive species in the Great Lakes in the late 1980’s and established (quagga mussels) in Lake Mead in 2007.

**Imhoff cone.** A clear, cone-shaped container marked with graduations, used by RISL to settle plankton tow samples as part of the process to analyze samples for early detection or enumeration of dreissenid mussel veligers.

**Veliger.** The microscopic, planktonic, larval stage of a dreissenid mussel.

**Plankton tow sample.** Water sample collected by towing or pulling a net and cod end made of a very fine mesh that filters water and retains any planktonic organisms living in the water.



## Appendix A – Table of SOP Comparisons

	Reclamation Invasive Species Lab, 2022	Western Regional Panel on Aquatic Nuisance Species, 2020	Alberta Agriculture and Forestry Water Quality Section, 2018	"British Columbia Ministry of Environment and Climate Change Strategy, 2018	California Department of Fish and Wildlife, 2017	MT Fish, Wildlife, & Parks, 2019
<b>Sampling Technique</b>	64 µm plankton net	64 µm plankton net. Samples collected depending on depth of waterbody. Typically, water temp is greater than or equal to 9°C. Number of locations on each waterbody depends on size of waterbody, number of tows per site depends on water depth and net size. Recommends a minimum of 1000 L total volume sampled per sample site.	63 µm plankton net, samples obtained from no less than 1 M above canal bed. A horizontal upstream sweep was performed when flows were low	30-50 cm plankton net, sampled at sites with varying depths, samples between 9-12°C	63/64 µm plankton net, sites are sampled monthly when water temperatures are between 9-18°C. Tows at a depth of 0-15 M, with a minimum volume of 1000 L filtered per site	NA
<b>Field Blank Collected (Yes/No)</b>	Yes	Not mentioned	Not mentioned	Not mentioned	Not mentioned	NA

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	<b>Reclamation Invasive Species Lab, 2022</b>	<b>Western Regional Panel on Aquatic Nuisance Species, 2020</b>	<b>Alberta Agriculture and Forestry Water Quality Section, 2018</b>	<b>"British Columbia Ministry of Environment and Climate Change Strategy, 2018</b>	<b>California Department of Fish and Wildlife, 2017</b>	<b>MT Fish, Wildlife, &amp; Parks, 2019</b>
<b>Preservation Method (Tris/baking soda &amp; ethanol)</b>	Recommends Tris with baking soda option / EtOH, 70% by volume	Recommends Tris with baking soda option / EtOH, 70% by volume	Not specified	5% baking soda and 70% EtOH	4% baking soda solution and 20% volume EtOH	Baking soda, 95% EtOH at 70% volume, sometimes live samples
<b>Refrigeration &amp; storing</b>	Samples are stored in cooler during collection, then refrigerated until purged	Bottles are stored in cooler on ice upon collection. Bottles are refrigerated until samples are examined	Not specified	Unpreserved samples are stored on ice, preserved samples are not refrigerated and are stored in dark, cool place	Collected in cooler at time of collection. Refrigerated until shipping (time unspecified)	
<b>Settling/Cone setup</b>	Settled in modified Imhoff cones	Not specified	Not specified	Not specified	Not specified	No - samples are filtered
<b>Settling process (time, if bottles are shaken, how much is preserved)</b>	Samples are settled for 24 hours, bottom 15 mL collected and analyzed by microscopy	Not specified	Not specified	Not specified	Samples are sent to CDFW's Shellfish Health Lab, Bodega Marine Laboratory	None

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<b>Cross-contamination Preventions</b>	Known positive samples are set up in a separate area with dedicated equipment; nets are soaked in vinegar between sample sites; each waterbody has a dedicated net	Recommend dedicated nets. Decontamination between sites when a site is known positive or suspected to be positive. Decon between sampling trips required: vinegar soak (1-2 hours)/bleach spray (10-minute soak)	Not specified	Using vinegar, bleach, and freezing equipment to decontaminate	Vinegar soak (min 2 hours), 10% bleach (15 min); no mention of between sites/waterbodies	
<b>Debris management</b>	Large debris such as sticks, rocks, fish, etc., are removed during collection. Sandy or muddy tows are discarded and recollected.	Not mentioned	Samples are collected in smaller volumes in turbid and algae heavy conditions. Only 500 L are collected and filtered compared to 1000 L	No sampling after storms	Not specified	
<b>Cross-polarized Microscopy (yes/no)</b>	Yes	Discussed in a separate document	Not specified	Not mentioned	Yes	Yes, based on WRP
<b>PCR (yes/no)</b>	Yes, 40 mL aliquot collected from bulk water sample at time of login	Discussed in a separate document	Not specified	Not mentioned	Yes	No

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	<b>Reclamation Invasive Species Lab, 2022</b>	<b>Western Regional Panel on Aquatic Nuisance Species, 2020</b>	<b>Alberta Agriculture and Forestry Water Quality Section, 2018</b>	<b>"British Columbia Ministry of Environment and Climate Change Strategy, 2018</b>	<b>California Department of Fish and Wildlife, 2017</b>	<b>MT Fish, Wildlife, &amp; Parks, 2019</b>
Other info		States "...tows from the same waterbody may be composited into a single bottle"				FWP utilizes a risk prioritization model (FWP AIS Early Detection and Monitoring Protocols) to assign a risk category; samples are then processed according to this priority with the goal of analyzing high-risk samples faster

